Double blind trial of recombinant human erythropoietin in preterm infants

A J B Emmerson, H J Coles, C M M Stern, T C Pearson

Abstract

Twenty four infants between 27 and 33 weeks' gestation were recruited into a double blind study to investigate the use of recombinant human erythropoietin (r-HuEpo) for the prevention of anaemia of prematurity. Between 50 and 150 U of r-HuEpo (n=16) or placebo was administered subcutaneously twice a week from 7 days of age until discharge. There was a significant increase in the reticulocyte count in infants receiving r-HuEpo sustained from the second week of treatment until discharge compared with placebo. There was a reduction in the number of transfusions required in the r-HuEpo group with only 47% requiring a transfusion compared with 87% in the placebo group. During treatment with r-HuEpo there was a significant rise in the red cell folate concentration, a significant fall in the ferritin concentration, and a significantly higher percentage of haemoglobin F at discharge suggesting active erythropoiesis. The study provides strong evidence for the efficacy of r-HuEpo in stimulating erythropoiesis and reducing the requirement for transfusions for anaemia of prematurity.

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Anaemia of prematurity presents at around 6 weeks of age and has an increased incidence and is more profound with greater prematurity.[1] The aetiology of this anaemia is not clearly defined, although there is evidence for a disproportionately low endogenous erythropoietin concentration in anaemic preterm infants compared with anaemic adults or children.[2] The reason for the low erythropoietin concentration in the presence of anaemia has not yet been elucidated, although it might relate to increased oxygen delivery to the tissues after delivery[3] or to an insensitivity of the regulation mechanism.[4] There is evidence from cell culture studies of erythroid precursors from anaemic preterm infants that there are adequate numbers of both circulating[5 6] and marrow resident[7] precursors which are responsive to exogenous erythropoietin. Anaemia of prematurity presenting at around 6 weeks of age must be clearly separated from the early anaemia seen in sick premature infants during the first weeks of life. This early anaemia has many causes, the requirement for frequent blood sampling for monitoring being the commonest.[8] The consequences of anaemia of prematurity are significant, with tachycardia, tachypnoea, apnoea and poor feeding, leading to a requirement for blood transfusion.[9] There are well recognised complications of blood transfusion that include infection with cytomegalovirus[10] and the suppression of natural erythropoiesis after transfusion with adult haemoglobin, which leads to a worsened anaemia.[11]

After the cloning of the erythropoietin gene in 1985,[12] leading to the availability of recombinant human erythropoietin (r-HuEpo), it has been postulated that r-HuEpo may be useful for the prevention or treatment of anaemia in premature infants. Early pilot studies in anaemic preterm infants have suggested that r-HuEpo may indeed have a stimulatory effect on red cell production, with a rise in the reticulocyte count, though the efficacy of r-HuEpo in reducing the need for transfusion has not yet been proved.[13] In many of these studies, r-HuEpo treatment has been initiated when the infant was already anaemic, and a significant reduction in the number of transfusions was not shown. In the current study it was postulated that early administration of r-HuEpo would stimulate erythropoiesis before the development of anaemia of prematurity. In addition the early use of erythropoietin would reduce the number of transfusions required. Therefore we undertook a double blind placebo controlled study to investigate the safety and efficacy of r-HuEpo for the prevention of anaemia of prematurity, commencing the r-HuEpo treatment or placebo at around 7 days of age.

Subjects and methods

Signed informed consent was obtained during the first week of life from one or both parents of 24 infants (10 boys, 14 girls) born between 27 and 33 weeks' gestation. Infants were excluded from the study if they showed signs of a primary haematological disease, any clinically significant intrauterine infection, a severe congenital abnormality, a persistent systolic blood pressure of greater than 60 mm Hg, or had had seizures.

During the first week of life baseline haematological investigations were undertaken, which included a full blood count, ferritin, vitamin B-12, and red cell folate concentrations. In addition, baseline urea and electrolyte measurements and urinanalysis were performed. These investigations were repeated immediately before the infant was

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discharged from hospital, being one to three days after the last dose. Sixteen infants eligible for entry into the study were randomised to the r-HuEpo group (Eprex, Cilag Ltd) and eight to the placebo group (4% albumin solution) with a deliberate 2:1 double blind randomisation. The first nine infants received 50 U/kg, the next nine infants received 100 U/kg, and the final six infants received 150 U/kg of r-HuEpo or placebo twice a week subcutaneously into the buttock commencing after 7 days of age. r-HuEpo or placebo was administered until the infant was discharged home.

After each injection basic observations of the heart and respiratory rates, along with the temperature and blood pressure, were made and note was taken of any reaction to the study treatment. During the inpatient stay weekly measurements of the full blood count (Coulter S-plus), reticulocyte count (supravital stained film), and electrolytes (DAX 96) were made as is the normal policy on our unit. The ferritin (Becton Dickenson), vitamin B-12 (Becton Dickenson), and red cell folate (Becton Dickenson) concentrations were evaluated every two weeks. Haemoglobin F measurements were made by the method of Philips *et al.*[14] In addition, at termination, blood was taken for erythropoietin antibodies (Bio Scientia).

The decision to give a blood transfusion to a study infant was made by the medical staff of the neonatal unit who were blinded to the randomisation. The unit policy at the time of the study was to transfuse a preterm infant who had a haemoglobin concentration less than 100 g/l and who had symptoms consistent with those caused by anaemia. The symptoms and signs of anaemia included poor feeding, tachycardia, tachypnoea, apnoea, and pallor. Infants with a haemoglobin less than 80 g/l were transfused even if asymptomatic. All infants received multivitamins and folic acid (0·2 mg) from 2 weeks of age and iron (6·25 mg) in the form of ferrous glycine sulphate from 4 weeks of age.

Throughout the study period, a record was taken of the number of transfusions given, the volume of blood transfused, and the amount of blood withdrawn for investigations. Follow up of the infants was carried out for one year, but only the first three months' follow up data are presented.

Statistical analysis was by Student's t test for normally distributed data and the Mann-Whitney U and χ^2 tests for other data. Because of the small numbers of infants in each of the dose groups, the three dose groups were combined for all statistical analyses. Ethical approval for the study was obtained from the St Thomas's Hospital ethics committee.

At the outset of the study it was calculated that for a reduction in transfusions of 50% to be statistically significant at the 5% level with a power of 85%, 36 infants should be recruited. However, due to the unexpected deaths of two infants from sudden infant death syndrome (SIDS, see results and discussion) the study was terminated prematurely after 24 infants had been recruited. This inevitably has reduced the power of the study particularly for the parameter of the number of transfusions given to each group.

Results

Twenty four infants were recruited to the study, with 16 in the r-HuEpo treated group and eight in the placebo group. There were no significant differences between the r-HuEpo and placebo groups for any variable (table 1) before the study. Twenty three infants completed the study, the other infant (number 3) was randomised to the r-HuEpo treatment group but was withdrawn from the study at 14 days of age to enable transfer to another hospital for maternal reasons. The data for this infant have not been included in the statistical analyses. Fifteen infants received a mean (SE) of 10·2 (0·9) doses each of 96 (10·3) U/kg of r-HuEpo.

There was a significantly higher mean reticulocyte count in the r-HuEpo group of 106×10^9/l after week 2 ($p<0·01$) when compared with the placebo group at 58×10^9/l. The difference (95% confidence interval) between the means of the two groups was 48×10^9/l (19 to 75×10^9/l). This difference was sustained until the administration of r-HuEpo was stopped at discharge (fig 1). The highest mean reticulocyte count in the r-HuEpo and placbo groups were the pretreatment values (120 and 119×10^9/l). The reticulocyte count in the r-HuEpo group remained between 100 and 110×10^9/l being 1·8 times that of the placebo group ($50–60\times10^9$/l). After termination of treatment the mean reticulocyte counts did not differ significantly between the r-HuEpo and placebo groups at any stage during the three months' follow up (fig 1).

There was a significant fall in the mean ferritin concentration in the r-HuEpo treated group by 84 μg/l (19 to 149 μg/l) to a mean of 88 μg/l compared with the placebo group mean of 172 μg/l at termination ($p<0·02$) of the active treatment phase (fig 2). There was a rapid decline in the mean ferritin from a mean pretreatment concentration of 214 μg/l to 101 μg/l over the first four weeks until supplementation with iron was commenced. The rate of decline in the mean ferritin in the r-HuEpo group then slowed, though continued to fall and reached 88 μg/l at discharge. In the placebo group, the mean ferritin fell by

Table 1 Variables measured in 24 preterm infants studied before starting treatment. Results are mean (SE)

	r-HuEpo (n=16)	*Placebo (n=8)*
No. of boys	5	5
Gestational age (weeks)	29·9 (0·3)	30·4 (0·6)
Birth weight (g)	1363 (63)	1502 (97)
Apgar score (5 min)	9·1 (0·3)	9·3 (0·3)
Age at first dose (days)	9 (0·4)	8 (0·6)
Haemoglobin (g/l)	157 (7)	157 (7)
Packed cell volume	0·47 (0·02)	0·46 (0·02)
Haemoglobin F (%)	89 (2)	91 (1)
Reticulocytes ($\times10^9$/l)	120 (30)	119 (25)
Neutrophils ($\times10^9$/l)	5·63 (1·46)	3·48 (0·44)
Platelets ($\times10^9$/l)	293 (35)	303 (58)
Ferritin (μg/l)	206 (33)	213 (55)
Vitamin B-12 (mmol/l)	880 (144)	945 (193)
Red cell folate (μg/l)	733 (38)	808 (105)
Systolic blood pressure (mmHg)	55·3 (1·6)	51·0 (3·0)

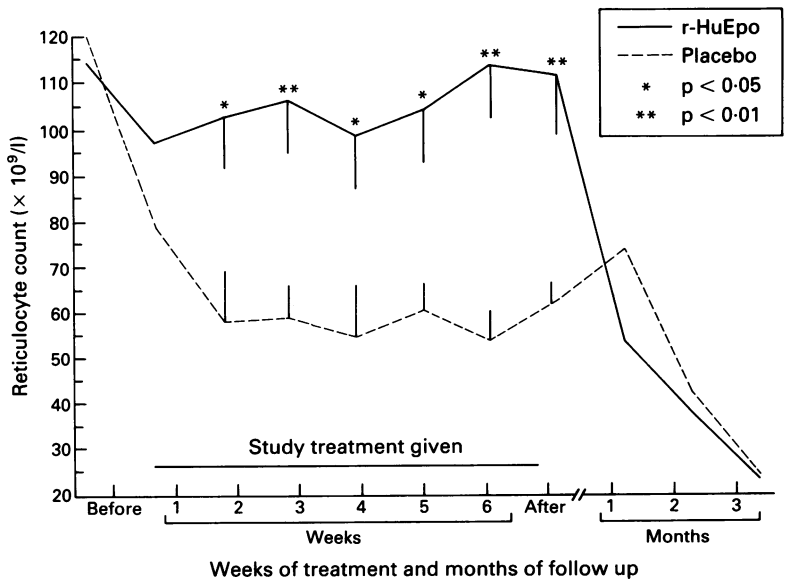

Figure 1 The mean (SE) reticulocyte counts during treatment with subcutaneous r-HuEpo (n=15) or placebo (n=8) in preterm infants between 27 and 33 weeks' gestation during six weeks of treatment and three months of follow up. Significance of the r-HuEpo group against the placebo group is shown. After=1–3 days after cessation of treatment.

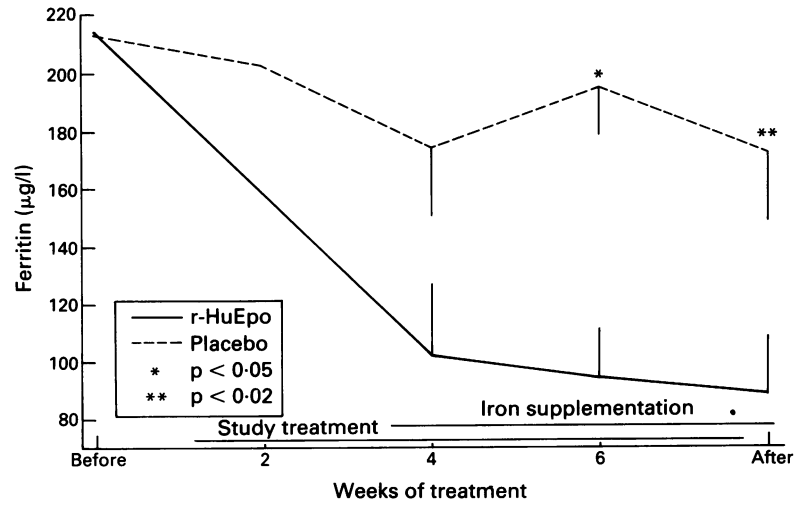

Figure 2 The mean (SE) serum ferritin concentrations in preterm infants between 27–33 weeks' gestation treated with r-HuEpo (n=15) or placebo (n=8). Iron supplementation (6·25 mg) from 4 weeks of age. Significance of the r-HuEpo group against the placebo group is shown. After=1–3 days after cessation of treatment.

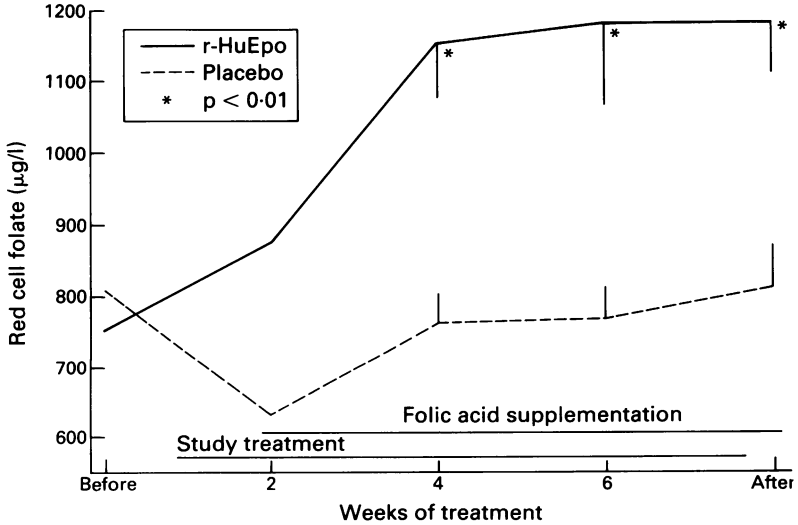

Figure 3 The mean (SE) red cell folate concentrations in preterm infants between 27–33 weeks' gestation treated with r-HuEpo (n=15) or placebo (n=8). Folic acid (0·2 mg) was supplemented from 2 weeks of age. Significance of the r-HuEpo group against the placebo group is shown. After=1–3 days after cessation of treatment.

38 µg/l to a mean of 174 µg/l and then remained constant after iron supplementation (fig 2). No infant was iron deficient at any stage as measured by the ferritin concentration.

After supplementation with folic acid, there was a significant increase in the mean red cell folate concentration by 390 µg/l (177 to 603 µg/l) ($p<0·01$) in the r-HuEpo treated group to a mean of 1150 µg/l (fig 3), being 51% higher than in the placebo group at 760 µg/l from week 4 onwards (fig 3).

The difference in the fall of the percentage fetal haemoglobin at termination of the treatment compared with that pretreatment between the two groups was 19% (0·1 to 38·7%) ($p<0·05$). There was a significantly higher concentration of fetal haemoglobin at the end of the study in the r-HuEpo group of 69% compared with the placebo group of 52% ($p<0·05$).

The number of infants requiring one or more transfusions was lower in the r-HuEpo treated group, with 47% requiring transfusions with a maximum of two, while 88% of the placebo group required transfusions, the maximum being three. The difference (95% confidence interval) between those needing a transfusion in each of the r-HuEpo or placebo groups at 41% (–1 to 83%; $p=0·14$), although large, does not reach statistical significance. A greater mean volume of blood was transfused in the placebo group, 19·6 ml/kg, compared with the r-HuEpo treated group, 10·0 ml/kg, the difference being 9·6 ml (–1·6 to 20·6 ml) does not reach statistical significance.

The mean systolic blood pressure in the r-HuEpo treated group of 81·9 mm Hg was greater than in the placebo group at 75·7 mm Hg by 6·2 mm Hg (–3·6 to 16·0 mm Hg) at termination, which was not significant (table 2). At termination, the mean platelet count in the r-HuEpo treated group was higher by 51×10^9/l (–55 to 158×10^9/l) at 436×10^9/l compared with 385×10^9/l for the placebo group. The mean neutrophil count in the r-HuEpo treated group at termination, $2·3\times10^9$/l, was lower than the placebo group, $3·1\times10^9$/l, by $0·8\times10^9$/l (–0·4 to $1·9\times10^9$/l) (table 2). No infant had developed antibodies to r-HuEpo at termination of the study. The weight increase in the r-HuEpo treated group at week 6, 21·1 g/day, was greater by 1·3 g/day (–3·6 to 6·2 g/day) than the placebo group at 19·8 g/day.

During the study period and the first three months of follow up after discharge, there was no significant difference in the haemoglobin concentrations between the two groups, although the mean haemoglobin in the placebo group was below that for the r-HuEpo group for the first four weeks until blood transfusions were given. In addition, there was no evidence for a sudden fall in haemoglobin once administration of r-HuEpo had ceased.

ADVERSE REACTIONS

There were some adverse reactions during the treatment phase, but none could be ascribed to the r-HuEpo treatment (table 3). After

discharge home, after the study medication had ceased, three deaths occurred, all of these infants had received r-HuEpo. One infant (number 6) developed acute volvulus of the gut two days after discharge home, and at laparotomy was found to have infarcted most of the large and small bowel. This infant had received 100 U/kg/week r-HuEpo. The other two infants (numbers 2 and 22) both died four weeks after discharge from hospital, and after a postmortem examination the cause of death was ascribed to SIDS. One infant received 100 U/kg/week and the second received 300 U/kg/week of r-HuEpo.

Discussion

Concern over the use of blood transfusions in preterm infants, and the frequent development of anaemia in otherwise well infants, has prompted the investigation of alternative treatments.[15] Although the endogenous concentrations of erythropoietin in preterm infants is low,[16–18] there appear to be adequate erythroid precursors both in the marrow and in the peripheral circulation, which are responsive to r-HuEpo as demonstrated by cell culture techniques.[5–7] It has been the practice in many units to wait until an infant with anaemia is symptomatic before blood transfusions are given.[19 20] The use of r-HuEpo from an early stage might reduce the pathologically low haemoglobin concentrations seen in preterm infants, producing a situation mimicking the physiological fall in haemoglobin seen in term infants.[1]

Our study clearly demonstrates that the use of subcutaneous r-HuEpo, twice a week from 1 week of age, leads to a sustained increase in the reticulocyte count compared with the placebo group. In addition, the significantly increased concentration of red cell folate incorporated into the newly formed red cells in the r-HuEpo treated group, and the dramatic and significant fall in the ferritin concentration, collectively suggest the presence of active erythropoiesis in the r-HuEpo treated group compared with the placebo group. The fact that the highest reticulocyte counts in either of the r-HuEpo or placebo groups were found in the pre-treatment period just after birth is consistent with the understanding that endogenous erythropoietin is released during acute hypoxic stress at the time of delivery.[21]

The reduction in the number of infants requiring one or more transfusions from 88% in the placebo group compared with 47% in the r-HuEpo treated group, along the significantly higher percentage haemoglobin F at termination of the study, provides further support for the efficacy of r-HuEpo in stimulating erythropoiesis and reducing the need for blood transfusions in this patient group. The power of this study to demonstrate statistical significance for this large reduction in the need for transfusion is reduced due to the unexpected termination of the study after the deaths of the two infants from SIDS.

During the study period the mean haemoglobin concentration for the placebo group remained below that for the r-HuEpo treated group until week 4 (fig 4). The apparent increase in the mean concentration at week 5 for the placebo group and at week 6 for the r-HuEpo group are due to blood transfusions given to the infants in each group. A greater percentage of infants in the placebo group (88%) were given transfusion compared with the r-HuEpo group (47%), which has resulted in the mean haemoglobin concentrations between the groups not being significantly different.

The reported doses of r-HuEpo used so far have ranged from 37·5 U/kg twice a week[13] to 700 U/kg in an alternate daily regimen,[22] with several studies suggesting that there is a rise in the reticulocyte count.[13 22 23] One double blind study used intravenously administered r-HuEpo and did not show any significant difference in the reticulocyte count, nor in the number of transfusion between the r-HuEpo and placebo groups.[24] It has been reported that doses of 700 U/kg/week lead to an increase in the packed cell volume from 0·28 to 0·30 over a three week period. It is, however, still not clear what the optimal dose or frequency of administration of r-HuEpo should be.

In several studies, the r-HuEpo treatment has been commenced when the infant was

Table 2 Variables measured in 23 preterm infants studied at the termination of treatment. Results are mean (SE)

	r-HuEpo (n=15)	*Placebo (n=8)*
Haemoglobin (g/l)	108 (5)	111 (12)
Packed cell volume	0·32 (0·02)	0·33 (0·03)
Haemoglobin F (%)	69 (4·7)	52 (5·9)*
Reticulocytes ($\times 10^9$/l)	111 (10)	62 (7)**
Neutrophils ($\times 10^9$/l)	2·3 (0·2)	3·1 (0·7)
Platelets ($\times 10^9$/l)	436 (29)	385 (43)
r-HuEpo antibodies	None	None
Blood removed (ml/kg)	25·5 (2·6)	26·5 (4·6)
Volume transfused (ml/kg)	10·2 (3·0)	19·2 (5·3)
Days to discharge	44·9 (3·2)	47·4 (6·0)
Systolic blood pressure (mmHg)	81·9 (2·6)	75·7 (4·3)
Weight increase (g)	840 (80)	730 (90)
Weight increase (g/day)	22·3 (1·6)	51·0 (3·0)

*p<0·05; **p<0·01.

Table 3 Number and type of adverse and concomitant events seen during and after treatment in 23 preterm infants studied

	r-HuEpo (n=15)	*Placebo (n=8)*
Events during treatment		
Cardiorespiratory:		
Apnoea/bradycardia	4	4
Patent ductus arteriosus		1
Chest infection	1	1
Gastrointestinal:		
Abdominal distension	1	
Bloody stools	1	
Vomiting		1
Constipation		1
Haematology/biochemical:		
Biochemical rickets	3	
Low neutrophil count		1
Skin rashes:		
Nappy rash	2	1
Others:		
Lethargy		
Oedematous legs	2	1
Ophthalmia neonatorum	3	
Hernia	1	
Events during follow up		
General:		
Wheezing	1	
Infection	3	
Hernia	1	1
Rash		1
Deaths:		
Volvulus	1	
SIDS	2	

several weeks old, or when the infant was already anaemic , in an attempt to prevent the need for transfusion.[13 24] It may be that the delayed use of r-HuEpo has not provided sufficient time to allow correction of the anaemia before the onset of symptoms precipitated the use of transfusion. Our study commenced the treatment after the first week and even earlier use of r-HuEpo in the first few days may well increase the efficacy.

For safety reasons we chose to commence with a low dose of 100 U/kg/week, rising to 300 U/kg. There has been evidence from several sources that the use of erythropoietin in preterm infants may result in the development of neutropenia. Halpérin *et al* showed a fall in the neutrophil count, reaching a nadir at 56 days after r-HuEpo therapy at a dose of 75–300 U/kg/week,[13] and Ohls and Christensen showed neutropenia in the peripheral blood and reduced number of matured stored neutrophils in the bone marrow with doses of 700 U/kg/week.[22] This was not found in the study of Shannon *et al*,[24] although they found a significant inverse correlation with the value of the reticulocyte count in both the r-HuEpo and placebo groups. Although the absolute neutrophil count was slightly lower in the r-HuEpo treated group in our study this was within the normal range and was not significantly different from the placebo group, and no correlation was found between the reticulocyte and neutrophil counts. There have also been conflicting results from in vitro work, with some reports of reduced granulocyte production associated with the use of r-HuEpo,[25] while others have not found this.[26]

The deaths of two infants from SIDS one month after the termination of the active treatment in our study raises concern over the relationship with the use of r-HuEpo. The hypothesis that there may be development of a profound anaemia once the r-HuEpo treatment has ceased is not supported by the follow up evidence of haemoglobin concentrations (fig 4). There was no evidence that any infant became anaemic after cessation of treatment.

A second hypothesis, that r-HuEpo may cause persistence of haemoglobin F and that, under adverse circumstances, there may be compromised delivery of oxygen to the tissues leading to death, is based on the tenous evidence that SIDS may be associated with persistence of haemoglobin F.[27] This association of SIDS with haemoglobin F has, however, not been found by all investigators.[28 29] The two infants who died from SIDS in our study had lower haemoglobin F concentrations at termination of treatment than the mean for the r-HuEpo group and were more consistent with the placebo group, all being lower than the natural concentration of a mean of 72% in healthy term infants.[30] There have been no other deaths due to SIDS associated with the use of r-HuEpo in any of the published studies.

This study clearly shows active erythropoiesis and a reduction in the use of blood transfusions for the anaemia of prematurity with the use of modest doses of r-HuEpo, although further studies with larger numbers of infants are required to clarify the optimal dose, frequency, and starting time for the prevention of this anaemia.

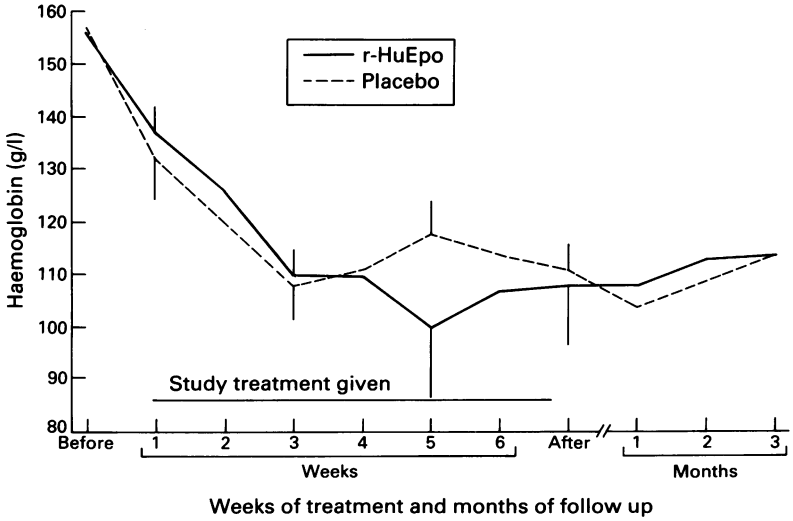

Figure 4 The mean (SE) haemoglobin concentrations for preterm infants between 27 and 33 weeks' gestation treated with r-HuEpo (n=15) or placebo (n=8) during six weeks of active treatment and three months of follow up.

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